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14. ABSTRACT
The focus of this research project was to characterize the nature of JP-8 toxicity to the skin and continue development of an in vitro model system, membrane coated fiber (MCFG) array, for assessing physiochemical parameters related to hydrocarbon partitioning and absorption through skin. In vitro studies with human epidermal keratinocytes demonstrated that inhibition of the NF-κB pathway with blockers confirms its role in cytokine production in jet fuel and hydrocarbon exposure in vitro. This could potentially reduce the inflammatory effect of fuel exposure in vivo. Exposure to the synthetic hydrocarbon fuel S-8 also is capable of inducing epidermal keratinocyte irritation in vitro as assessed by cytotoxicity and cytokine release. We have shown close correlation between MCF predicted dermal absorption of a series of compounds and measured permeability in skin. Patterns of aromatic hydrocarbon partitioning into three different MCF fibers (Polydimethylsiloxane, Polyacrylate, Carbowax) from three different vehicles (water, water/ethanol, biological albumin containing media) were different and could serve as a basis for clustering jet fuel hydrocarbon constituents in interpreting their patterns of absorption or biodistribution.

15. SUBJECT TERMS
Jet fuel, skin, dermal toxicity, analytical chemistry, partition coefficients, chemical mixtures, JP-8, Jet A, S-8

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EXECUTIVE SUMMARY:

All of the research supported under the present agreement have been published (or in press at the time of this report) in peer-reviewed journals (see list below) and thus are freely accessible over public library networks (e.g. Pub Med). A component of the present grant was to complete and publish data from the two previous AFOSR grants (F49620 -98-1-0105 and -01-1-0080) that formed the basis for many of the studies conducted. This report will focus on briefly summarizing the major findings of this research program relative to jet fuel dermatotoxicity and assessing physical chemical parameters of jet fuel hydrocarbons as they relate to dermal penetration using a physical in vitro system specifically developed for this purpose.

Introduction and Background:

The focus of this research project was to characterize the nature of JP-8 toxicity to the skin and continue development of an in vitro model system for assessing physiochemical parameters related to hydrocarbon partitioning and absorption through skin. JP-8 consists of hydrocarbon-rich kerosene base commercial jet fuel (Jet-A) plus additives DC1-4A, Stadis 450, and diethylene glycol monomethyl ether. Previous studies {best reviewed in *Muhammad F, Monteiro-Riviere NA, Riviere JE: Comparative in vivo toxicity of topical JP-8 jet fuel and its individual hydrocarbon components: Identification of tridecane and tetradecane as key constituents responsible for dermal irritation. Toxicologic Pathology 33: 258-266, 2005*} confirmed the hypothesis that mid-chain aliphatic hydrocarbons (e.g. tridecane and tetradecane) may be primarily responsible for dermal irritation seen with topical exposure to jet fuel. Differences between dermal irritation seen between Jet-A, JP-8 and JP-8(100) may be due to additives modifying the absorption of these hydrocarbons from the jet fuel, and not a result of direct toxicity from the additives. Thus, as is seen with pharmaceutical formulations, vehicle effects which increase partitioning between the fuel and the lipids of the stratum corneum epidermal skin barrier will enhance delivery. Thus,
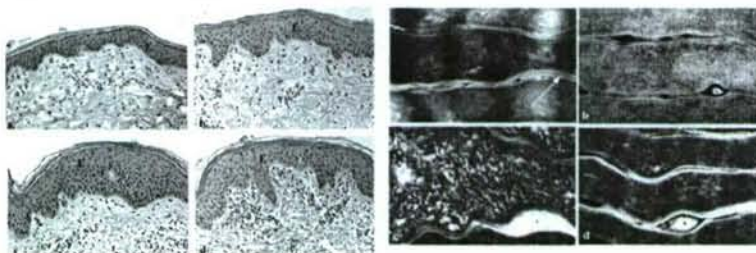
aliphatic hydrocarbons may paradoxically be delivered better from a mixed fuel such as JP-8 or

JP-8 + 100 compared to Jet A, or even potentially a pure aliphatic fuel such as the synthetic S-8

(studies not conducted), underscoring the principle that exposure and delivery of a toxin must

first occur before toxicity can ensue. These concepts were fully developed in the previous Final

Report and the resulting publications, and are outlined in the summary inserts below.

Comparison of *In Vitro* and *In Vivo* Effects of JP-8 Aliphatic Hydrocarbons

Data demonstrates that Tridecane and Dodecane have maximal *in vivo* effects (microabscesses, erythema, ↑thickness). These compounds are not uniquely cytotoxic *in vitro* but are on the border of being well absorbed (Tridecane) versus retained in stratum corneum (SC) based on FTIR.

Hydrocarbon	Absorption	[SC] FTIR	Micro-abscess 1day	Macro-Erythema 4day	Epidermis Thickness (µ) 1day	Epidermis Thickness (µ) 4day	*In Vitro* Cytotoxicity 24 h	*In Vitro* IL-8 24 h
Undecane (C11)	+	-	-	++	56	64	75%	++++
Dodecane (C12)	+	-	-	++	56	63	65%	++
Tridecane (C13)	+	-	+	++++	85	79	70%	+++
Tetradecane (C14)	-	+	+/-	++++	75	82	50%	+++
Pentadecane (C15)	-	+	+/-	+++/ ++++	68	70	35%	+
Hexadecane (C16)	-	+	-	++	61	61	30%	+

Morphological evidence in support of long-chain aliphatic hydrocarbons producing *in vivo* response similar to JP-8
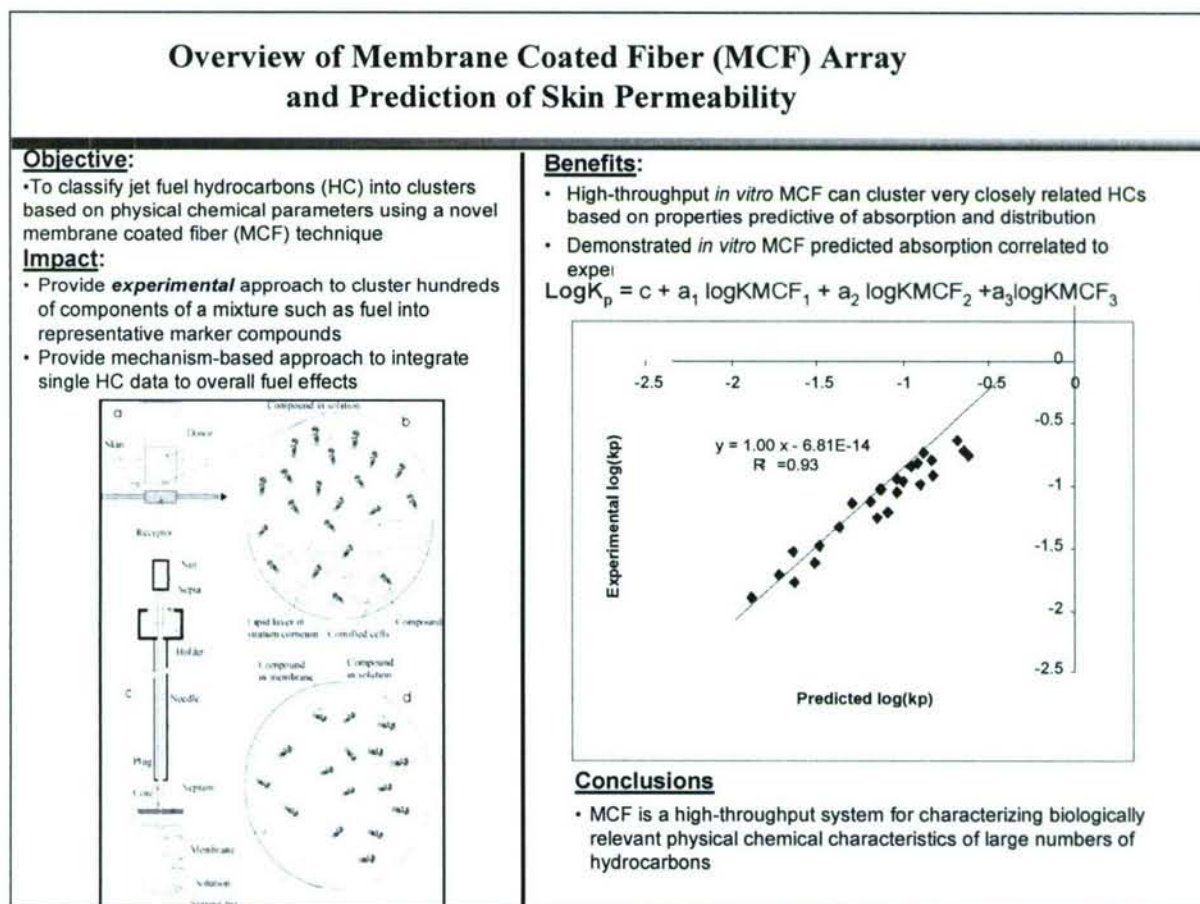

Skin following 1-day exposures to (a) control; (b) JP-8; (c) tridecane; (d) tetradecane. Note increase in dermal inflammation with aliphatic HC. Epidermis (E). x145.

Stratum Corneum lipid layers after 1-day exposures to: (a) control; (b) JP-8; (c) o-xylene; (d) tetradecane. Note expanded intercellular spaces (*) and similarity of tetradecane to JP-8 (TEM x 69,700 - ruthenium tetroxide)

These studies led to our research focus in two areas: continued development of an in vitro method that could be used to dissect out vehicle and chemical mixture effects on partitioning and absorption of component hydrocarbons, and human epidermal keratinocyte (HEK) studies on cellular effects of fuels and aliphatic hydrocarbons, as well as These data will be reviewed below.

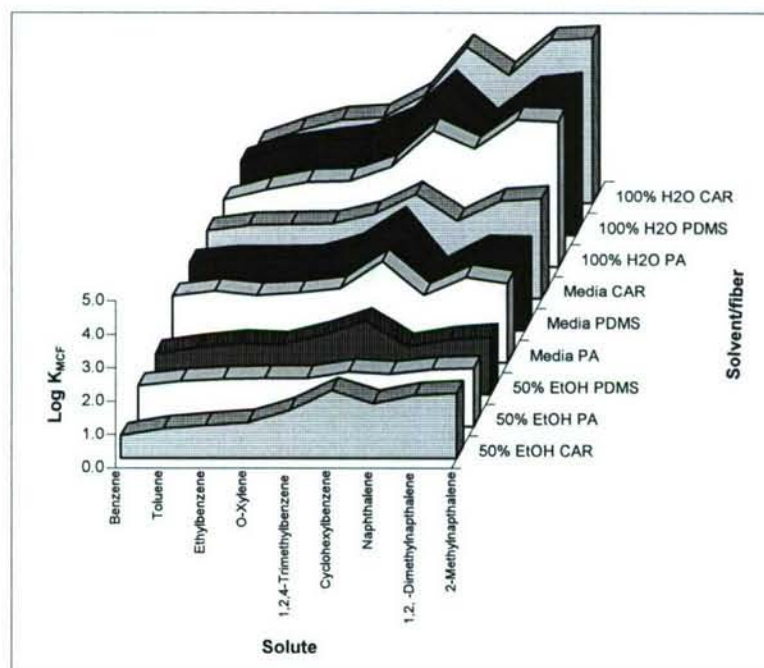**Membrane Coated Fiber (MCF) Studies:**

During the course of this research, a novel physical chemical approach was used to characterize chemical partitioning from a vehicle to a membrane. This method, fully described in the manuscripts listed below (Xia et al; Baynes et al.), and is also outlined in the summery insert.

Overview of Membrane Coated Fiber (MCF) Array and Prediction of Skin Permeability

<u>**Objective:**</u>
•To classify jet fuel hydrocarbons (HC) into clusters based on physical chemical parameters using a novel membrane coated fiber (MCF) technique

<u>**Impact:**</u>
• Provide *experimental* approach to cluster hundreds of components of a mixture such as fuel into representative marker compounds
• Provide mechanism-based approach to integrate single HC data to overall fuel effects

<u>**Benefits:**</u>
• High-throughput *in vitro* MCF can cluster very closely related HCs based on properties predictive of absorption and distribution
• Demonstrated *in vitro* MCF predicted absorption correlated to expe

$$LogK_p = c + a_1 logKMCF_1 + a_2 logKMCF_2 + a_3 logKMCF_3$$

$y = 1.00 x - 6.81E-14$
$R = 0.93$

(Experimental log(kp) vs Predicted log(kp))

<u>**Conclusions**</u>
• MCF is a high-throughput system for characterizing biologically relevant physical chemical characteristics of large numbers of hydrocarbons

These studies demonstrated that knowledge of the partitioning of a chemical into three diverse MCF can accurately predict the same chemicals absorption through skin. This suggests

that the technique can be used to classify chemicals based on measured properties that are now known to related to dermal absorption based on this research (Xia et al., 2007).

In our most recent publication (Baynes et al., 2007), the partitioning of 9 aromatic components of jet fuel into three MCFs (Polydimethylsiloxane - PDMS, Carbowax, Polyacrylate) from three vehicles (water, 50% water-ethanol, albumin buffered media) was assessed. As seen with other studies, ethanol reduced partitioning into fibers based on its ability to increase solubility of the aromatic hydrocarbons in vehicles. Albumin also reduced partitioning presumably due to its solubilizing effects. These studies confirm the overarching effect of vehicle on membrane partitioning and provide an experimentally clean and high-throughput system for determining partitioning coefficients of a wide variety of chemicals from diverse vehicles. Secondly, the pattern of a chemical's partitioning across three fibers provides a characterization an individual molecule's behavior in biological systems. This is illustrated in the differential patterns below.

Similar patterns have been generated for aliphatic hydrocarbons and will be reported shortly. These patterns clearly differentiate individual hydrocarbon patterns of partitioning from different vehicles. These MCF patterns could potentially be used to cluster chemicals to predict absorption or tissue biodistribution in physiological based pharmacokinetic (PBPK) models. Finally, the physical-chemical interaction data imbedded in such a pattern will be adapted to characterize nanomaterial interactions in biological milieu.

Keratinocyte Cell Culture Studies:

These studies were designed to study the potential signal transduction pathways involved in jet fuel and aliphatic hydrocarbon induced dermal irritation, initially focused on inhibitors of the NF-κB pathway. HEK were exposed to JP-8, synthetic aliphatic hydrocarbon fuel S-8, and either pentadecane, tetradecane, tridecane, and undecane. Additional studies were conducted with signal transduction pathway blockers parthenolide (3.0μM), isohelenin (3.0μM), SB 203580 (13.3μM), substance P (3.0μM), and recombinant human IL-10 (10ng/ml). In the absence of inhibitors, JP-8 and to a lesser extent undecane and S-8, had the greatest toxic effect on cell viability and inflammation suggesting, as least *in vitro*, that synthetic S-8 fuel is less irritating than currently used JP-8. Each inhibitor significantly ($p<0.05$) decreased HEK viability. DMSO, the vehicle for parthenolide, isohelenin, and SB 203580, had minimal effect on viability. Overall, IL-8 production was suppressed at least 30% after treatment with each inhibitor. Parthenolide was the most effective inhibitor of IL-8 release; IL-8 was significantly decreased after exposure to undecane, tridecane, tetradecane, and pentadecane but significantly increased after JP-8 exposure compared to controls. Inhibitors were not effective in suppressing IL-8 release in JP-8 exposures to control levels. These data suggest that inhibiting NF-κB pathway, which appears to play a role in cytokine production in HC-exposed HEK *in vitro*, may reduce the inflammatory

effect of HC *in vivo*. The potential toxicity of S-8 compared to JP-8 is difficult to assess in these in vitro studies, since as discussed above, absorption of the aliphatic hydrocarbons from S-8 will determine ultimate Toxicologic effects. However, these studies do demonstrate that dermal irritation is possible with S-8 and appears similar to that seen with either JP-8 or aliphatic hydrocarbons.

Transition / Technology Transfers:

- The MCF approach has been adopted to be used to describe the physiochemical nature of nanomaterials in situ that has served as the basis for a number of grant proposals.

Honors / Awards / Highlights:

- Doctor of Science (*honoris causa*), Purdue University, May 12, 2007.

- *Lifetime Achievement Award*, European Association of Veterinary Pharmacology and Toxicology, presented at the 10th EAVPT Congress, Turin, Italy, September, 2006.

- *2005 Elanco Distinguished Lecturer*, Elanco/ Eli Lilly, Indianapolis, IN, November, 2005.

- *10th Annual Clarenburg Lecturer*, Kansas State University, September, 2004.

Publications supported by the grant which formed the basis of this report:

1. Muhammad F, Baynes RE, Monteiro-Riviere NA, Xia XR, Riviere JE. Dose related absorption of JP-8 jet fuel hydrocarbons through porcine skin with QSPR analysis. Toxicol. Mechanisms Methods. 14: 159-166, 2004.

2. Muhammad F, Brooks JD, Riviere JE. Comparative mixture effects of JP-8 (100) additives on the dermal absorption and disposition of jet fuel hydrocarbons in different membrane model systems. Toxicology Letters. 150: 351-365, 2004.

3. Monteiro-Riviere NA, Inman AO, Riviere JE. Skin toxicity of jet fuels: Ultrastructural studies and the effects of substance P. Toxicol. Appl. Pharmacol. 195: 339-347, 2004.

4. Xia XR, Baynes RE, Monteiro-Riviere NA, Riviere JE. A compartment model for the membrane-coated fiber (MCF) technique used for determining the absorption parameters of chemicals into lipophilic membranes. Pharm. Res. 21:1345-1352, 2004.

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6. Xia XR, Baynes RE, Monteiro-Riviere NA, Riviere JE. Determination of the partition coefficient and absorption kinetic parameters of chemicals in a lipophilic membrane/water system by using a membrane coated fiber technique. Eur. J. Pharm. Sci 24: 15-23, 2005.

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11. Xia XR, Baynes RE, Monteiro-Riviere NA, Riviere JE: An experimental based approach for predicting skin permeability of chemicals and drugs using a membrane coated fiber array. Toxicol. Appl. Pharmacol. 221: 320-328, 2007.

12. Xia XR, Baynes RE, Monteiro-Riviere NA, Riviere JE. A system coefficient approach for quantitative assessment of the solvent effects on membrane absorption from chemical mixtures. SAR and QSAR Environ. Res. 18: 579-593, 2007

13. Baynes RE, Xia XR, Barlow B, Riviere JE: Partitioning behavior of aromatic components in jet fuels across diverse membranes. J. Toxicol. Environ. Health (In Press).

14. Inman AO, Monteiro-Riviere NA, Riviere JE: Inhibition of jet fuel hydrocarbon induced toxicity in human epidermal keratinocytes. J. Appl. Toxicol. (In Press).

Published abstracts based on this grant:

1. Xia XR, Riviere JE: Differentiation of the absorption of jet fuel hydrocarbons with an ethanol/water system and a membrane-coated fiber technique. Toxicological Sci. 78 (S1): 2004.

2. Riviere JE, Xia XR: Uptake kinetic of jet fuel aromatic hydrocarbons from aqueous solutions studied by a membrane coated fiber technique. Toxicological Sci. 78 (S1): 2004.

3. Muhammad F, Monteiro-Riviere NA, Riviere JE: Topical hydrocarbon absorption in porcine skin previously exposed to JP-8 jet fuel. Toxicological Sci. 78 (S1): 2004.

4. Riviere JE, Monteiro-Riviere NA, Xia XR, Muhammad F. Continuing studies on the interaction of JP-8 and its constituent hydrocarbons with the skin. AFOSR Jet Fuel Toxicology Workshop. Tucson, AZ. October, 2004.

5. Basak SC, Riviere JE, Baynes RE, Xia XR, Gute BD. A hierarchical QSAR (HiQSAR) approach to predict dermal penetration of JP-8 chemicals. AFOSR Jet Fuel Toxicology Workshop. Tucson, AZ. October, 2004.

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16. Basak S, Riviere JE, Baynes RE, Gute BD, Witzman FA: Predicting skin penetration and interaction with JP-8. <u>AFOSR Jet Fuel Toxicology Workshop,</u> Tucson, AZ, 2007.

17. Riviere JE, Baynes RE, Xia XR, Monteiro-Riviere NA. Determination of biologically relevant physical chemical parameters of JP-8 hydrocarbons using the membrane coated fiber array. AFOSR Jet Fuel Toxicology Workshop, Tucson, AZ, 2007.